Journal of Medical Virology 59:146–153 (1999)

Selection of Genetic Variants of the 5′ Noncoding Region of Hepatitis C Virus Occurs Only in Patients Responding to Interferon α Therapy

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Interferon α (IFN α) can suppress the replication of hepatitis C virus (HCV) in chronically infected patients. However, HCV persists in a significant number of patients despite the normalization of alanine transaminase (ALT) during IFN α therapy. In this study, HCV variants in patients under IFN α therapy were characterized to examine their role in viral persistence during the therapy. Sixteen patients selected for this study were infected with HCV genotype 1b and remained HCV RNA positive for at least 1 month after onset of therapy. Nine patients responded to the therapy in terms of normalization of ALT (responders), whereas seven patients did not show a significant decrease of ALT level (nonreseponders). To examine HCV populations in these patients, the HCV 5′ noncoding region (5′ NCR) was analyzed by polymerase chain reaction amplification and sequencing. Newly emerging variants of the HCV 5′ NCR replaced predominant variants present prior to IFN α therapy in six of nine responders. Most predominant HCV variants during IFN α therapy carried a nucleotide substitution G to A at nt 231 within the 5′ NCR. An analysis of the HCV quasispecies population in one responder revealed that a preexisting variant became predominant under IFN α therapy. These results emphasized the importance of the genetic heterogeneity of the HCV genome for viral resistance to IFN α. Five of seven HCV isolates from nonresponders were identical to those found in responders with regard to the nucleotide sequence of the 5′ NCR. However, no selection of variants of the HCV 5′ NCR occurred in nonresponders during the course of therapy. We conclude that IFN α treatment leads to the selection of variants of the HCV 5′ NCR only in responders and may act differently in nonresponders. Our results suggest that the HCV 5′ NCR may be a target of anti-HCV actions of IFN α. ***J. Med. Virol. 59:146–153, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: HCV; variants; selection by IFN α

INTRODUCTION

Interferon α (IFN α) eliminates hepatitis C virus (HCV) in only 15–30% of chronic infected patients [Di Besceglie et al., 1989; Hoofnagle, 1994; Iino et al., 1994]. Genetic determinants on the HCV genome may contribute to the viral resistance to IFN α. First, the responder rate to IFN α therapy depends on HCV genotype [Kanai et al., 1992; Hino et al., 1994; Mita et al., 1994; Tsubota et al., 1994; Fried and Hoofnagle, 1995; Kohara et al., 1995]. Second, IFN α treatment leads to dramatic changes in the HCV population in individual patients, indicating different susceptibilities to IFN α of HCV quasispecies in a single patient [Hagashi et al., 1993; Enomoto et al., 1994; Mizokami et al., 1994; Yun et al., 1996; Gonzalez-Peralta et al., 1997; Lu et al., 1997; Polyak et al., 1998]. Genetic determinants of HCV conferring resistance to IFN α have been under intensive investigation. It was suggested that a high variability within the hypervariable region 1 (HVR1) of the HCV E2 region may correlate with the status of nonresponders [Okada et al., 1992; Yeh et al., 1996; Le Guen et al., 1997; Polyak et al., 1998]. However, the selection of the HCV E2 region by the immune response does not appear to be a determining factor for the response to IFN α therapy [Sakuma et al., 1996]. Recently, the correlation of the genetic variation of a region called interferon sensitivity determining region (ISDR) within the HCV NS5A with the response to IFN α therapy is under controversial discussion [Enomoto et al., 1995, 1996; Chayama et al., 1997; Khorsi et al., 1997; Zeuzem et al., 1997; Rispeter et al., 1998]. Interestingly, the HCV NS5A is able to repress an IFN α-in-

Grant sponsor: Fritz-Bender-Stiftung.

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Accepted 1 January 1999

duced protein kinase PKR through direct interaction with the protein kinase catalytic domain [Gale et al., 1997, 1998].

The current studies to understand the IFN α resistance of HCV are focused mainly on the characterization of HCV variants in nonresponders. However, this approach faces the uncertainty whether IFN α actually does induce an antiviral status in nonresponders. Therefore, a more rational approach would be to examine HCV variants replicating in patients responding to IFN α therapy (responders). A normalization of serum alanine transaminase (ALT) and a decrease of serum concentration of HCV RNA in responders indicate the suppression of HCV replication by IFN α. However, HCV RNA remained detectable in a significant number of responders despite the normalization of ALT during IFN α therapy [Lau et al., 1993b; Aiyama et al., 1994; Hino et al., 1995; Chemello et al., 1996]. Therefore, at least some HCV variants can resist the antiviral activities of IFN α and maintain the viral replication in responders. Previously, we examined changes in HCV quasispecies populations in IFN α-treated patients by a newly established method termed *heteroduplex analysis* [Lu et al., 1997]. New HCV variants with nucleotide substitutions within the core and E1 region were found in both responders and nonresponders, whereas a predominant variant of the HCV 5′ noncoding region (5′ NCR) emerged in a responder during IFN α therapy. These results indicate that a selection of HCV variants occurred in responders. In this report, we selected nine responders and seven nonresponders who remained HCV RNA positive during IFN α therapy and characterized HCV variants from these patients by reverse transcription-polymerase chain reaction (RT-PCR) amplification and direct sequencing of the 5′ NCR. HCV quasispecies populations in serial samples from one responder were analyzed by heteroduplex analysis and sequencing of clones of the 5′ NCR.

PATIENTS AND METHODS

Patients

Sixteen chronically HCV-infected patients included in the present study were treated with recombinant IFN α 2a (Essex) between 1991 and 1993 and were under clinical surveillance until 1996. All patients were selected by the criterion that HCV RNA was detectable by RT-PCR in at least one serum sample during the therapy. HCV RNA level, histologic activity index (grading), and development of fibrosis (staging) according to Knodell et al. [1981] were determined before the beginning of therapy (Table I). Twelve patients received 5 million units IFN α 2a daily for 4 months and then 5 million units three times a week for an additional 8 months. Four patients interrupted therapy after 5–10 months due to severe side effects. Three sustained responders (SR1–3) and six partial responders (PR1–6) showed a normalization of ALT after onset of the therapy. SR 1–3 remained HCV negative in a follow-up period of 24 months after cessation of therapy. PR1–6 suffered from relapse after the end of therapy.

TABLE I. Clinical Description of the Selected Patients

Patient	Age[a] (years)	HCV RNA level (10^5 GE/ml[b]) before therapy	HCV RNA level (10^5 GE/ml[b]) after therapy	Histologic activity index (HAI)[c] Grading	Histologic activity index (HAI)[c] Staging
SR1	36	<3.5	—	4	0
SR2	37	n.d.	—	4	0
SR3	35	<3.5	—	2	0
PR1	48	<3.5	56.7	5	2
PR2	41	7.0	10.0	3	0
PR3	45	<3.5	n.d.	6	0
PR4	39	6.5	60.1	2	0
PR5	31	19.9	9.4	2	0
PR6	52	<3.5	n.d.	4	1
NR1	40	<3.5	50.0	3	1
NR2	62	9.8	n.d.	5	1
NR3	64	<3.5	70.7	8	2
NR4	62	158.4	265	6	4
NR5	52	35.1	107	n.d.	n.d.
NR6	41	104.0	n.d.	4	0
NR7	51	n.d.	n.d.	6	0

HCV, hepatitis C virus; n.d., not determined.
[a]Age at the beginning of the IFN treatment.
[b]GE/ml: HCV genome equivalent per ml serum defined by using Quantiplex HCV-RNA 1.0 assay (Chiron).
[c]Histologic activity index scoring [Knodell et al., 1981].

Seven patients were nonresponders (NR1-7) because a normalization of ALT did not occur in these patients under IFN α therapy. These patients had no marker of chronic hepatitis B virus and human immunodeficiency virus (HIV) infection.

Quantitation of HCV RNA in Patient Sera

HCV RNA titer in patient sera was quantified by the Quantiplex HCV-RNA 1.0 Assay (Chiron, Emeryville, CA) following the manufacturer's instructions. The detection limit of this assay is 3.5×10^5 HCV genome equivalents (GE)/ml.

Extraction of HCV RNA, RT-PCR, Cloning, and Sequencing of PCR Fragments

Extraction of HCV RNA was carried out as described previously [Lu et al., 1995]. All patients included in this study were infected by HCV genotype 1b. Primers derived from the sequence of the HCV genotype 1b prototype J1 were used for RT and PCR [Kato et al., 1990]: sense primers p10 5′-GGC GAC ACT CCA CCA TAG AT-3′ (nt 6–25), p15 5′-ACC ATA GAT CAC TCC CCT GT-3′ (nt 17–36), and antisense primers p11 5′-GAT GCA CGG TCT ACG AGA CC-3′ (nt 329–310), p16 5′-CAA GCA CCC TAT CAG GCA GT-3′ (nt 295–276). RTs were carried out by using antisense primer p11. Ten microliters of extracted HCV RNA were reverse transcribed by 2.5 μM primer and 200 units of recombinant moloney murine leukemia virus reverse transcriptase (BRL Life Technologies, Gaithersburg, MD) for 60 min at 37°C. cDNA fragments were amplified by nested PCR using primers p10/p11 for the first run and primers p15/p16 for the second run. PCR was performed from 5 μl cDNA over 30 cycles including denaturation at 94°C for 30 sec, annealing at 50°C for 60

TABLE II. Detection of HCV RNA in Patients During IFN Therapy

Patients	Duration of therapy (months)	HCV RNA detected in months	HCV RNA negative in months	HCV RNA reappeared in month	HCV RNA status after 2 years
SR1	12	0,1,4,8,11	12		–
SR2	12	0,7			–
SR3	12	0,1	5		–
PR1	12	0,5,12,>12			+
PR2	6	0,2,4,511,>12			+
PR3	12	0,7,>12			+
PR4	12	0,1	5,12	>12	+
PR5	5	0,2	5	10	+
PR6	10	0,4	10	>12	+
NR1	12	0,4,10,>12			+
NR2	12	0,1,4,8,12,			+
NR3	12	0,1,4,8,>12			+
NR4	12	0,1,4,8,>12			+
NR5	12	0,6,9,>12			+
NR6	12	0,2,6,>12			+
NR7	8	0,3	8	12	+

HCV, hepatitis C virus; IFN, interferon.
+, positive for HCV RNA; –, negative for HCV RNA defined by RT/PCR. The detection limit of PCR used for this study is 2×10^3/ml as determined by using the NIBSC standard for HCV.

sec, and extension at 72°C for 120 sec. The PCR products, 278-bp fragments, were examined by agarose gel electrophoresis. PCR fragments were subjected to direct sequencing by using primers 15 or 16. If necessary, PCR fragments were cloned into a commercially available vector pCRII (InVitrogen, San Diego, CA) following the manufacturer's instruction. Plasmid purification and agarose gel electrophoresis were carried out as described by Sambrook et al. [1989]. Sequencing was performed using an A.L.F. sequencer (Pharmacia, Schweden) or by a commercial service (Tetlow, Germany).

Identification of HCV Variants by Heteroduplex Analysis Using Temperature Gradient Gel Electrophoresis (TGGE)

HCV variants in a quasispecies population were characterized as described previously [Lu et al., 1995, 1997]. HCV RNA was extracted from patient serum; the HCV 5′ NCR were amplified by RT-PCR. Because these PCR products consisted of heterogenous fragments derived from different HCV variants, we generated a number of independent clones of amplified cDNA fragments; plasmids containing the cDNA fragments were purified and used as template in PCR with primers 15 and 16 to generate DNA fragments for heteroduplex analysis, as described below. The TGGE was performed following the manufacturer's instruction (Diagen, Hilden, Germany) with minor modifications. Samples for TGGE were prepared as follows: 2 μl PCR products (1 μg DNA) were mixed with 2 μl reference DNA fragment and 2 μl sample buffer (0.5 M MOPS pH 8, 4 M urea), heated at 94°C for 5 min and reannealed at 40°C for 15 min. In all experiments described here 8% polyacrylamide gels (acryamide stock 30:08, 20 mM MOPS pH 8.0, 8 M urea, 2% glycerol, 0.03% ammonium persulfate) were used. The temperature gradient ranged between 35 and 60°C. The gel was run at 300 V for 3 hr, then stained with 1 μg/ml ethidium bromide for 10 min and examined under ultraviolet (UV) light. Different HCV variants showed distinguishable patterns in TGGE. For heteroduplex analysis of cDNA clones of the HCV 5′ NCR in this study, a clone V4.3 containing an uncommon nucleotide substitution A to G at nt position 205 was used as a reference DNA fragment.

RESULTS

Presence of HCV RNA in Patients Treated With IFN α

Nine patients who responded to IFN α therapy remained HCV RNA positive for different durations after the onset of therapy (Tale II). Three sustained responders (SR1, SR2, and SR3) were negative HCV RNA in the follow-up period of more than 2 years. Three partial responders (PR1–3) remained HCV RNA positive by RT-PCR detection at all time points during the study despite the normalization of ALT. HCV RNA became transiently undetectable by RT-PCR in three partial responders (PR4, PR5, and PR6) under IFN α therapy and reappeared after the cessation of therapy. In six of seven nonresponders (NR1–6), HCV RNA was detectable during the whole period of therapy. Patient NR7 was negative for HCV RNA after 8 months of therapy but interrupted therapy and became HCV RNA positive in month 12. HCV RNA titers in samples of six responders and four nonresponders were quantified by the bDNA assay (Table I). SR1 and SR3, PR1, and PR3 had continuously low serum level of HCV RNA under 3.5×10^5 HCV GE/ml, the detection limit of the quantitation assay. The initial HCV RNA level in PR2, PR4, and PR5 was 7.0×10^5, 6.5×10^5, and 19.9×10^5 GE/ml, respectively. The HCV RNA level in these patients decreased transiently under 3.5×10^5 GE/ml. A transient reduction of HCV RNA titer was observed in NR4 and NR5 during therapy. Many patients had significantly

A

```
94          105   170                       192   230            244
TGCAGCCTCC AG  -  ACGACCGGGT CCTTTCTTGG ATC  -  CGAGACTGCT AGCCG

CR1
.......... ..  -  .......... .......... ...  -  .......... .....   0
.......... ..  -  .......... .......... ...  -  .......... .....   1
.......... ..  -  .......... .......... .T.  -  .......... .....   4
.......... ..  -  .......... .......... ...  -  .......... ....A   8
.......... ..  -  .......... .......... .A.  -  .A........ .....  11

CR2
.......... ..  -  .......... .......... ...  -  ......C... .....   0
.......... G.  -  .......... .......... .T.  -  .......... .....   7

CR3
.......... ..  -  .......... .......... ...  -  .......... .....   0
.......... ..  -  .......... .......... ...  -  .......... .....   1

PR1
.......... ..  -  .......... .......... ...  -  .......... .....   0
.......... ..  -  .......... .......... ...  -  .......... .....   5
.......... ..  -  .......... .......... ...  -  .......... .....   7
.......... ..  -  .......... .......... ...  -  .A........ .....  12
.......... ..  -  .......... .......... ...  -  .......... ..... >12

PR2
.A........ ..  -  .......... .......... ...  -  .......... .....   0
.A........ ..  -  .......... .......... ...  -  .......... .....   2
.A........ ..  -  .......... .......... ...  -  .......... .....   4
.A........ ..  -  .......... .......... ...  -  ......C... .....   5*
.A........ ..  -  .......... .......... ...  -  .......... .....  11
.A........ ..  -  .......... .......... ...  -  .......... ..... >12

PR3
.......... ..  -  .T........ .......... ...  -  .......... .....   0
.......... ..  -  .......... .......... ...  -  .A........ .....   7
.......... ..  -  .......... .......... ...  -  .A........ ..... >12

PR4
.......... ..  -  .......... .......... ...  -  .......... .....   0
.......... ..  -  .......... .......... ...  -  .A........ .....   1
.......... ..  -  .......... .......... ...  -  .A........ ..... >12

PR5
.......... ..  -  .......... .......... ...  -  .A........ .....   0
.......... ..  -  .......... .......... ...  -  .A........ .....   2
.......... ..  -  .......... .......... ...  -  .A........ .....  10
.......... ..  -  .......... .......... ...  -  .A........ ..... >12

PR6
.......... ..  -  .......... .......... ...  -  .A........ .....   0
.......... ..  -  .......... .......... ...  -  .A........ .....   4
.......... ..  -  .......... .......... ...  -  .A........ ..... >12
```

Figure 1

B

```
94          105   170                        192   230            244
TGCAGCCTCC AG  -  ACGACCGGGT CCTTTCTTGG ATC  -  CGAGACTGCT AGCCG
                                                                  Month
NR1*
.......... ..  -  .......... .......... ...  -  .......... ..... 0,4,10,>12

NR2
.......... ..  -  .......... .......... ...  -  .A........ ..... 0,1,4,8,>12

NR3
.......... ..  -  .......... .......... ...  -  .......... ..... 0,1,4,8,>12

NR4
.......... ..  -  .......... .......... ...  -  .......... ..... 0,1,4,8,>12

NR5
.......... ..  -  .......... .......... ...  -  .......... ..... 0,6,9,12

NR6
.......... ..  -  .......... .......... ...  -  .......... ..... 0,2,6,12

NR7
.......... ..  -  .......... .......... ...  -  .......... ..... 0,3,12

*: A nucleotide substitution T to A at nt 163 was found in the isolate from
NR1.
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Fig. 1. Predominant variants in patients treated by interferon α (IFN α). **A:** Predominant variants in responders were characterized by reverse transcription-polymerase chain reaction (RT-PCR) and direct sequencing at different time points during therapy. The nucleotide sequences of relevant parts nt 94–105, 170–192, and 230–244 of the hepatitis C virus (HCV) 5′ noncoding region (NCR) are shown. The numbering of the nucleotide sequence is according to HCV genotype 1b prototype J1 [Kato et al., 1990]. **B:** Predominant variants in nonresponders were characterized by RT-PCR and direct sequencing at different time points during the therapy.

higher HCV RNA titers after IFN α therapy (Table I). ALT raised in these patients at the end of the therapy when the relapse occurred.

Emergence of New Predominant HCV Variants of the 5′ NCR in Responders During IFN Therapy

To examine the change of HCV populations in IFN α-treated patients, the nucleotide sequences of amplified cDNA fragments comprising the HCV 5′ NCR were determined by direct sequencing. The sequence data are summarized in Figure 1. New predominant variants of the HCV 5′ NCR emerged in six responders (SR1, SR2 PR1, PR2, PR3, and PR4). Four different predominant variants were found in serial samples of SR1 before HCV RNA in serum became undetectable. In SR2, HCV RNA was not detected at month 3 but reappeared at month 7 with three nucleotide substitutions within the 5′ NCR. New variants became predominant in responders PR1 and PR2 during IFN therapy. However, the original variants took over again after therapy. In contrast, new predominant variants emerged in PR3 and PR4 and persisted even after therapy. No new variant of the HCV 5′ NCR was identified in three patients (SR3, PR5, and PR6) during IFN α therapy.

The predominant variants emerging during IFN α therapy differed from those prior to the therapy in a few nucleotides (Fig. 1). A transition G to A at nt position 231 occurred in four patients (SR1, PR2, PR3, and PR4), alone or together with other exchanges. Interestingly, predominant HCV variants with a nucleotide A at nt position 231 preexisted in two partial responders (PR5 and PR6) before therapy and no new variant was found during the IFN α therapy. The frequent occurrence of this nucleotide substitution suggests that variants with the nucleotide A at nt position 231 may have some selective advantages under IFN therapy. Two nucleotide substitutions at conserved positions, G to A at nt 244 and A to G at nt 104, were identified in SR1 and SR2, respectively. These two substitutions were not found before in other known HCV isolates.

No new predominant variant of the 5′ NCR was observed in nonresponders during the IFN α therapy. Preexisting predominant variants in nonresponders

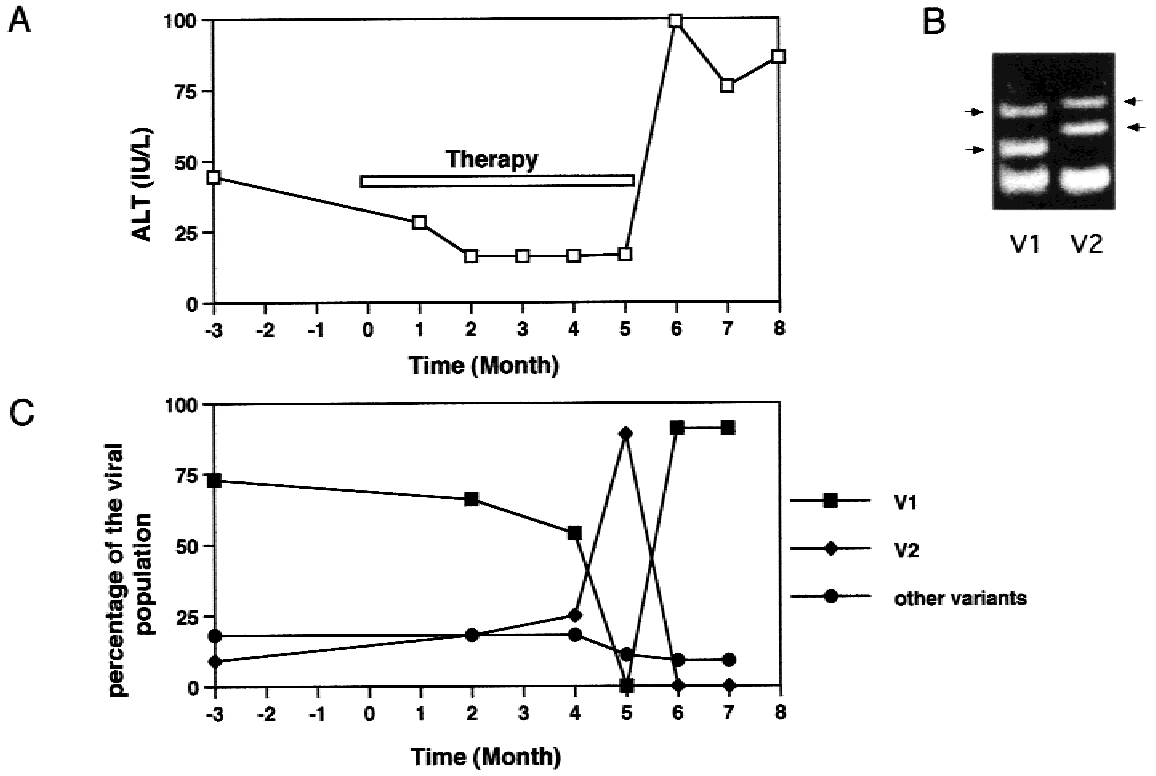

Fig. 2. Selection of a preexisting hepatitis C virus (HCV) variant by interferon α (IFN α). **A:** Alanine transaminase (ALT) level of responder PR2 during the course of IFN-α therapy. **B:** Identification of PR2.V1 and V2 by heteroduplex analysis in temperature gradient gel electrophoresis (TGGE). The patterns of heteroduplexes formed by the sample and the reference in TGGE are characteristic for each variant. The heteroduplexes are indicated by arrows. **C:** Composition of the HCV population in patient PR2 during the course of IFN α therapy.

persisted through the whole period of surveillance, including the therapy (Fig. 1B). The sequence analysis of the 5′ NCR of HCV isolates from six nonresponders revealed that NR1 and NR2 had nucleotide A at nt 163 and A at nt position 231, respectively. The 5′ NCR of the HCV isolates in five nonresponders (NR3–7) had the same nucleotide sequence as the variant preexisting in SR1 and PR1.

We examined the HCV 5′ NCR in serial samples of seven untreated patients and did not find new predominant variants during the same period of observation (data not shown).

Preexisting HCV Variants Can Be Selected by IFN α During Therapy

A large number of HCV variants coexist in chronically infected patients. Among 24 independent cDNA clones of the HCV 5′ NCR obtained from PR2 3 months before therapy (month −3), a predominant variant PR2.V1 occurred at a frequency of 70%. The nucleotide sequence of PR2.V1 is identical to the sequence generated by direct sequencing of the corresponding PCR product (Fig. 1). A coexisting variant PR2.V2 has a nucleotide substitution T to C at nt 236 that is identical to the sequence generated by directed sequencing of the PCR product from month 5 (Fig. 1). Obviously, PR2.V2 became the predominant variant during IFN therapy. We determined the proportion of both variants V1 and V2 in patient PR2 during the therapy whereby a normalization of ALT was observed (Fig. 2A). V1 and V2 were distinguishable by TGGE as described in Patients and Methods section above (Fig. 2B). The frequency of PR2.V2 gradually increased during the therapy, whereas PR2.V1 disappeared completely in month 5 of the therapy (Fig. 2C). As shown in the previous section, the variant V1 took over again when the therapy ceased and ALT raised again.

DISCUSSION

We found that new or preexisting HCV variants of the 5′ NCR became predominant in six of nine responders during IFN α therapy. After the cessation of the therapy, these selected variants in two patients PR1 and PR2 were replaced by predominant variants that were present before therapy. These results indicate that particular variants of the HCV 5′ NCR have selective advantages under IFN α therapy but some are only favored by IFN α. In contrast, a selection of genetic variants of the HCV 5′ NCR by IFN α did not take place in nonresponders.

The response to IFN α is not correlated with the presence of particular variants of the 5′ NCR, because HCV isolates from sustained or partial responders (SR1, SR3, PR1, PR3, and PR4) and nonresponders (NR3–7) have the identical nucleotide sequence within the 5′ NCR. This finding is consistent with the results of Ya-

mamoto et al. [1997]. Yamamoto et al. examined the HCV 5′ NCR in 11 sustained responders and 14 nonresponders before the therapy. No association of sequence variation of the HCV 5′ NCR and efficacy of IFN therapy was found.

The IFN α-selected HCV variants in responders differed from the preexisting master variants at several positions of the nucleotide sequence. One of these nucleotide substitutions G to A at nt 231 of the HCV 5′ NCR was identified to be present in new variants isolated from four responders. The G/A variation at nt 231 exists naturally among HCV isolates. The majority of HCV genotypes 1a, 1c, 2a/b/c, and 6 possess A at this position, whereas G occurs preferentially in genotypes 1b, 3a/b/c, 4a, and 5 [Smith et al., 1995]. The HCV isolates with A at nt 231 preexisted in two responders (PR5 and PR6) and did not show any change within the 5′ NCR during the therapy. In addition, nucleotide substitutions at two usually well conserved positions (nt 244 and nt 104) were found in variants that emerged in patients SR1 and SR2. It is not known how these nucleotide substitutions within the HCV 5′ NCR confer selective advantages for HCV under IFN α therapy. The HCV 5′ NCR forms an internal ribosome entry site (IRES) that mediates the initiation of viral translation [Brown et al., 1992; Fukushi et al., 1994; Wang et al., 1994]. Nucleotide substitutions within the HCV 5′ NCR may influence the viral translation and its sensitivity to the antiviral actions of IFN α.

Indeed, IFN α leads to the activation of cellular protein kinase PKR in the presence of double-stranded RNAs, which phosphorylates the small subunit of the eucaryotic initiation factor eIF2-α [Hershey, 1989; Meuers et al., 1990]. The phosphorylation of eIF2-α inhibits initiation of translation and thereby interferes with viral replication. Gale et al. found that HCV NS5A can repress PKR by direct interaction [Gale et al., 1997]. The interaction of HCV NS5A with PKR and the repression of PKR require ISDR. Amino acid substitutions within ISDR may affect the function of NS5A and increased susceptibility of HCV to IFN α, as is being discussed controversially at the moment [Enomoto et al., 1995, 1996]. In this case, PKR may be not fully inhibited by ISDR with mutations and retain the activity to interfere with viral translation. These findings may explain the selective pressure on the HCV 5′ NCR in responders. ISDR of HCV isolates from some responders among our patients were found to carry one to two mutations in comparison with HCV 1b prototype [Rispeter et al., 1998]. The absence of a selective pressure on the HCV 5′ NCR in nonresponders may result from two reasons. HCV NS5A may block the antiviral action of IFN α, particularly the activity of PKR protein kinase, and abolish thereby the selective pressure on the HCV 5′ NCR. However, it is not excluded that the status of nonresponders results simply from the inability of the nonresponders to establish a proper antiviral state. The inducibility of cellular antiviral systems in individual patients may be a factor determining the responsiveness to IFN α and needs to be investigated in the future.

HCV forms heterogeneous quasispecies populations in infected patients [Martell et al., 1992]. We showed here that a preexisting HCV variant in a responder PR2 became predominant under IFN α therapy. Therefore, subpopulations of HCV may resist IFN α even when most of a viral population if IFN α-susceptible. Our results emphasize again that the high genetic variability of HCV can play an important role in drug resistance.

ACKNOWLEDGMENTS

We are grateful to Bettina Funsch and Sandra Luda for excellent technical assistance and Ulla Schmitz for DNA sequencing. We thank Fritz-Bender-Stiftung for financial support.

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